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CHOLINERGIC RECEPTOR SUBSTRATES OF NEURONAL PLASTICITY AND LEARNING (89-0046)

FINAL TECHNICAL REPORT

Project Period: 10/1/88 - 9/30/91

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I. SUMMARY

This project is part of an ongoing experimental analysis of the neural mediation of learning and memory. The overall objective is to document the learning-relevant dynamic physiological changes in brain circuit activities that mediate discriminative avoidance learning in rabbits. Electrophysiological multichannel recording of neuronal activity during learning in the behaving animal is a principal methodology. Important information is also provided by selective lesion-induced disruptions of neuronal circuit activity and behavior. The specific thrust of this project was collaborative, combining behavioral neurophysiology and receptor biochemistry in order to document learning-relevant changes in neurotransmitter receptor binding correlated with learning-relevant neuronal activity. In addition, hypotheses of a theoretical model of the task-relevant information flow and neural circuit/network interactions were evaluated. In each of four behaviorally defined stages of acquisition, a distinct topographic distribution pattern of training-induced cue-elicited neuronal excitation was documented by recording the training-related neuronal activity in five nuclei of the anterior thalamus and in the four layers of the posterior cingulate cortex. Topographic patterns of training-induced binding of M_2 acetylcholine and $GABA_A$ receptors in the anterior thalamus correlated with the stage-related topographic patterns of thalamic activity. Effects of hippocampal and mammillothalamic tract lesions and the properties of the topographic patterns themselves fostered the hypothesis that the patterns are a product of hippocampal efferent flow to cingulate cortex and anterior thalamus which is essential for context-specific mnemonic retrieval. Other findings demonstrated: a) an involvement of norepinephrine and serotonin turnover in anterior cingulate cortical learning-relevant processing; b) a contribution of cholingergic diagonal band of Broca projections to non-associative aspects of cingulate cortical functioning, and; c) interactions of hippocampal and AD thalamic neurons in relation to novelty-detection and novelty-induced suppression of ongoing behavior. These findings provide unprecedented progress in relation to identification of brain sites of synaptic plasticity, involved biochemical mechanisms and dynamic brain circuit activities underlying instrumental goal-directed learning.

II. GENERAL ORIENTATION

This section provides a brief overview of the project including a description of major findings obtained during the three-year project period. Citations by authors' names refer to the list of publications (Section VI) obtained since the project was initiated in the fall of 1988, concerning work supported by AFOSR. Citations using superscripts refer to the bibliography (Section V) at the end of the text.

The overall objective of this project is to understand the neural mediation of learning and memory processes. This goal is approached using the discriminative avoidance behavior of rabbits as a model system. Male albino rabbits acquire an instrumental conditioned response (taking a step in a wheel apparatus) to a 0.5-second tone (CS+) in order to avoid a foot-shock, delivered 5 seconds after tone onset. A different tone, a negative conditional stimulus (CS-), is presented as often as the CS+ but does not signal shock and thus does not call for a conditioned avoidance response. The CS+ and CS- are presented 60 times each in an irregular order every day until a learning criterion is reached. The criterion, which is attained after an average of three days of training, establishes asymptotic avoidance behavior (i.e., avoidance responses on an average of 85% of the CS+ trials and on fewer than 10% of the CS- trials).

Lesion studies have indicated that the cingulate area of the cerebral cortex and the "limbic" areas of the thalamus (the anterior and medial dorsal thalamic nuclei), which send axons to the cingulate cortex, are critically involved in the mediation of avoidance learning in several species. The specific involvement of these areas in mediating discriminative avoidance learning in rabbits has been confirmed (Gabriel, Sparenborg and Kubota, 1989; Gabriel, Kubota, Sparenborg, Straube and Vogt, 1991a).

Extensive multichannel electrophysiological recording of brain neuronal activity in these areas has demonstrated systematic changes, or plasticity, of the neuronal discharges elicited by the auditory cues during behavioral acquisition. Neuronal discharge magnitudes 15 - 500 milliseconds after tone onset increased during learning and became discriminative, i.e., of greater magnitude in response to the danger signal (the CS+) than to the safety signal (the CS-). In addition, a substantial proportion of the single neurons in cingulate cortex and in the projecting thalamic nuclei exhibited "pre-motor" firing ramps, i.e., a progressive build-up of firing frequency in trained rabbits during the interval between CS+ onset and the avoidance response, with peak firing incidence at 50 - 100 milliseconds before the response (Kubota, Poremba, Kang & Gabriel, 1991).

Additional analytic studies indicated that afferents from the cerebral cortex are not necessary for the training-induced activity in the limbic thalamus[7] (Gabriel, Vogt, Kubota, Sparenborg & Straube, 1991a) whereas afferents from the thalamus are essential for the expression of training-induced neuronal activity in cingulate cortex (Gabriel, Sparenborg & Kubota, 1989). These and other findings have fostered a theoretical model

of the basic circuit information flow underlying acquisition and performance of the avoidance behavior[5,6] (Gabriel, 1991). In brief, the model states that subcortical synaptic plasticity which develops during acquisition enables the CS+ to elicit, in limbic thalamus, greater firing frequencies than the CS-. The heightened discharges projected to cingulate cortex initiate the pre-motor firing of layer V cells, which is projected to the neostriatum to trigger the output of the locomotory avoidance response.

A remarkable property of the training-induced neuronal activity documented in these studies is its specificity, within various cytoarchitecturally distinct areas of cinglate cortex and limbic thalamus, to particular stages of behavioral acquisition. For example, work in the late 70's and early 80's demonstrated that training-induced discrimination between CS+ and CS- develops in the anterior cingulate cortex, medial dorsal thalamus and in the deep layers of posterior cingulate cortex during the earliest stages of behavioral acquisition, whereas the anterior ventral thalamic and upper layer posterior cingulate cortical neurons do not exhibit discrimination until behavioral learning has attained asymptote.[1,3,4] This cascade of training-induced changes was substantially elaborated during the three-year project period. The more recent studies demonstrated that neurons in each of five distinct anterior thalamic nuclei exhibited a peak of cue-elicited excitation in a different stage of behavioral acquisition, and distinct stage-related peaks were also seen in four layers of the posterior cingulate cortex which receive axonal input from the thalamic nuclei (Gabriel, Vogt, Poremba, Kubota & Kang, 1992) A detailed description of these results is provided in Section IV, C.

Receptor binding analyses demonstrated that the distinct peaks of excitation in the various thalamic nuclei are accompanied by increased binding of M_2 muscarinic receptors (Vogt, Gabriel, Vogt, Poremba, Jensen, Kubota & Kang, 1991), and peaks that develop in the earliest training stage in certain thalamic nuclei are accompanied by decreased binding of $GABA_A$ receptors (Vogt and Gabriel, Vogt & Kang, in preparation). These results implicate muscarinic and $GABA_A$ receptor regulation in the synaptic plasticity responsible for the thalamic training-induced activity, and they pave the way for future analyses of the involved biochemical mechanisms.

The brain's hippocampal formation, known to be importantly involved in higher-order processes of memory, communicates directly with the cingulate cortex and related thalamic nuclei via efferent projections from subfield CA1 of Ammon's horn to anterior cingulate cortex and from the subiculum to the posterior cingulate cortex and anterior thalamus.[12] A major line of inquiry of this project has concerned the functional significance of these hippocampal projections, as approached via studies of changes of behavior and training-induced unit activity in cingulate cortex and anterior thalamus produced by subicular and hippocampal lesions[7] (Gabriel, Vogt, Kubota, Sparenborg & Straube, 1991a; Kang & Gabriel, 1991).

In brief, the data indicate that hippocampal formation efferents limit or suppress the above-described training-induced neuronal excitation in limbic thalamus[7] (Gabriel

et al., 1991a). This suppressive modulation is not essential for acquisition or performance of the avoidance response in the standard training situation. Instead the suppression gives rise to training-stage-related topographic patterns of cue-driven excitation in limbic thalamus and cingulate cortex. Lesion studies indicate that the suppressive efferent influences from hippocampus (and from cingulate cortex) are essential for limiting or holding in check, the excitation levels of the thalamic cell groups during pre-, and post-peak training stages[7] (Gabriel et al., 1991a). In other words, these suppressive influences respresent hippocampal modulation responsible for the topographic patterning of stage-related excitation in limbic thalamus and cingulate cortex.

These results foster the hypothesis that the changing topographic patterns are necessary for adequate "retrieval specificity", i.e., the fact that learned behavior is called forth preferentially by the specific contextual stimuli present during original acquisition. This property is of utmost importance as it ties specific learned behaviors to specific learning contexts and thus limits interference, i.e., the retrieval of a memory/response in an inappropriate context.

A definitive evaluation of this hypothesis is proposed for the next project period. Confirmation would constitute a truly major breakthrough with regard to an essential memory mechanism of the brain and the first isolation of specific brain circuit activity underlying mnemonic retrieval.

III. ORIGINAL PROJECT OBJECTIVES

A. Detection of training-induced receptor binding correlated with training-induced neuronal activity

A major goal of this project was to capitalize on the property of training-stage specificity of the neuronal activity in cingulate cortex and limbic thalamus. Biochemical parameters found to exhibit training-stage specificity in parallel with stage-specific neuronal activity would constitute strong candidate biochemical substrates of the neuronal plasticity. This screening strategy could provide important clues to the fundamental biochemical and biophysical events underlying learning-relevant synaptic plasticity. This would be of great importance as it would allow an analysis of the biochemical bases of synaptic plasticities operating in brain circuitry having a specific, identified behavioral relevance.

The first goal of the project was thus to document training-induced regulation in learning-relevant brain areas of neurotransmitter receptors in relation to discriminative avoidance learning, and to relate receptor regulation to training-induced neuronal activity (TIA) in the involved areas.

1. Receptor assays

Rabbits were to be trained to various stages of discriminative avoidance acquisition [the first exposure (FE) to conditioning, the session of the first significant (FS) behavioral discrimination, the session of criterion (CRIT) attainment, overtraining (OT), or extinction (EXT, tone presentation without shock)], followed by sacrifice for assay of M_1 and M_2 muscarinic acetylcholine, $GABA_A$, and mμ and delta opoid receptors in layers and subfields of cingulate cortex and in the limbic thalamic nuclei. Certain experiments were to employ a pretraining (PT) control group given presentations of the tones to be used as conditional stimuli and the footshock unconditional stimuli in an unpaired manner. This procedure provides information about the effects of tone and shock stimulation in the absence of the critical pairing, essential for learning, of one of the tones with the shock. Two additional control groups were to be used in all studies; a) a naive control group given no tones or shock, and; b) a yoked control group that experienced the PT procedure with the unpaired shock presented at a decreasing incidence over consecutive days. The number of days and the decreasing incidence of shock were to be determined by the average values of these parameters during learning in a large group of rabbits.

2. Electrophysiological Studies

Neuronal activity was to be recorded in controls, in the assayed sites, and in the hippocampal formation, during acquisition, overtraining, extinction and sessions with unexpected stimuli. This aspect of the proposed work was intended to provide: a) documentation of training-induced activity in areas not yet examined (e.g., subdivisions of the AV thalamic nuclei, the AD and AM thalamic nuclei and in subfields and layers of the hippocampus and dentate gyrus); b) minimal redundant data collection in cingulate cortex in order to confirm past neuronal data under current experimental circumstances; c) documentation of neuronal processing of unexpected stimuli in these areas, in connection with objective III.

B. Localization of the training-induced receptor changes and TIA found in studies specified in objective I

1. Identification of the neuron classes which express receptor regulation in cingulate cortex.

Dissociated Cells. On the basis of pilot data indicating up-regulation of M_1 muscarinic acetylcholine receptors during learning, studies of the distribution of M_1 receptors on dissociated cingulate cortical cells were proposed. Receptor assays were to be performed in one hemishpere in three groups of rabbits given training to the PT, FE, or OT levels of acquisition.

Michael Gabriel

2. Assessment of the role of basal forebrain cholinergic projections in training-induced alterations of muscarinic receptors in cingulate cortex

a. Receptor assay. To determine whether cholinergic afferents are involved in plasticity-relevant receptor regulation in cingulate cortex, M_1 and M_2 cholinergic receptors were to be assayed in three groups of rabbits with bilateral electrolytic lesions of the diagonal band of Broca (DBB). Prior to the assay each group was to be trained to the FE, CR or OT levels of training.

b. Neuronal recording. In order to evaluate the contribution of the cholingergic projection to cingulate cortical training-induced neuronal activity, two additional groups with bilateral electrolytic lesions of the DBB or with bilateral ibotenic acid (IBO) lesions of the medial septum and recording electrodes in hippocampal/cingulate cortical subfields and in the limbic thalamic nuclei were to be studied during acquisition, overtraining and unexpected stimulus sessions. Control data were to be provided by results of the study specified in I.B.

3. Assessment of the thalamic influence on regulation of receptors in cingulate cortex and hippocampal TIA.

a. Receptor assay. To determine whether thalamic afferents are involved in training-induced receptor alterations in cingulate cortex, two experiments were to be performed with anterior or medial thalamic lesions. The rabbits were to be trained to the FE, CRIT or OT training levels.

b. Neuronal recording Two groups with either anterior or medial thalamic lesions were to receive training, overtraining and unexpected stimulus sessions. Neuronal activity was to be recorded in the hippocampal dentate gyrus, CA1, CA3, and dorsal/posterior subiculum. (Cingulate cortical but not hippocampal TIA had been documented previously in rabbits with limbic thalamic lesions). Control data were to be provided by results of the study specified in I.B.

4. Assessment of the subicular influence on regulation of receptor alterations in cingulate cortex and limbic thalamus

a. Receptor assay. To determine whether subicular afferents are involved in cholinergic or other training-induced receptor changes in cingulate cortex, rabbits with bilateral electrolytic lesions of the hippocampal subicular complex were to be trained to FE, CRIT or OT levels of avoidance learning and subsequently assayed for receptor changes.

b. Neuronal Recording. To determine whether the subicular lesions govern other properties of thalamic training-induced activity, neuronal records in limbic thalamic nuclei not previously studied in animals with lesions (AD, AM, MD) were to be obtained in a group of rabbits with subicular lesions. They were to receive training and unexpected stimulus sessions. Control data were to be provided by results of the study specified in I.B.

5. Lesions of the AD nucleus

Preliminary data replicated during this project period (Gabriel et al., 1991b) had demonstrated that massive training-induced excitation developed in the AD thalamic nucleus in the very first conditioning session, and this excitation declined during the continuation of training, as the excitation of anterior ventral thalamic neurons increased. These findings suggested a reciprocity between the AD and AV thalamic neurons, wherein neurons of the AD thalamic nucleus are involved in activating cingulate cortical neurons to suppress AV thalamic activity and the output of the learned behavior during novel early sessions of training and during exposure to other unexpected events and contingencies. This hypothesis was to be tested by recording the neuronal activity of the cingulate cortex and hippocampal formation and AV thalamic nucleus during training in a group of rabbits with bilateral electrolytic and IBO lesions in the anterodorsal (AD) thalamic nucleus, and in controls.

C. Identification of afferents controlling neuronal and cholinergic receptor plasticity in the limbic thalamic nuclei

Bilateral IBO lesions were to be made in the lateral dorsal tegmental nucleus (LDTN), to determine whether the resultant removal of cholinergic afferents will eliminate TIA in limbic thalamic nuclei. Controls were to receive sham lesions. Recordings were to be made in the anterior and medial dorsal thalamic nuclei in controls and in animals with lesions during acquisition and unexpected stimulus sessions.

IV. STATUS OF THE RESEARCH

A. Prologue

The proposed research was carried out in collaboration with Dr. Brent A. Vogt who, during August of 1990, moved his laboratory from the Boston University School of Medicine, Department of Anatomy to the Bowman Gray Medical School, Department of Physiology and Pharmacology, Winston-Salem North Carolina. The project at Illinois: a) supplied to Dr. Vogt brains of rabbits trained to various levels of avoidance learning

as specified above, and; b) documented TIA for correlation with alterations of receptor binding in key brain areas of known relevance to discriminative avoidance learning in rabbits; c) sought to identify the specific behavioral relevance, sources and mechanisms TIA.

The project in North Carolina performed the receptor binding assays in specified layers of cingulate cortex and nuclei in limbic thalamus.

B. Progress Toward the Objectives

1. Receptor Binding Studies.

a. Prologue. In pursuit of objective I,A, four major studies of training-induced alterations in receptor binding have been carried out using ligands for muscarinic M_1 and M_2, $GABA_A$, α_2, β_1, 5-HT_1, Mμ opoid, delta opiod and neurotensin receptors. Additional assays of nor-epinephrine (NE), serotonin (5-HT) and of metabolites of these neurotransmitters, 3-methoxy-4-hydroxy-phenyl-glycol (MHPG) and 5-hydroxy indoleacetic acid (5-HIAA), were carried out. The data were collected for these studies in the year preceding the first project year and during the first year. Assays and data analysis were performed in the first two project years. Here below the current status of this work is summarized.

The following training-induced changes in limbic thalamic and cingulate cortical binding of ^{3}H-oxotremorine (OXO), a ligand having high affinity for M_2 muscarinic receptors, are described in a manuscript by Vogt, Gabriel, Vogt, Poremba, Jensen, Kubota and Kang (1991), published in the Journal of Neuroscience. Manuscripts reporting training-induced changes in ligands specific to $GABA_A$ and opiod receptors, are in preparation.

b. OXO Binding in Limbic Thalamus. OXO binding increased significantly during training, in the parvocellular anterodorsal (ADp), the parvocellular anteroventral (AVp), and in the magnocellular AV (AVm) nuclei (Figure 1). Increased OXO binding in AVp paralleled the course of TIA development (reported below) showing a progressive increase over training stages, reaching maximum in the stage in which behavioral criterion was attained and declining during overtraining and extinction. OXO binding paralleled TIA in the ADp nucleus, in showing an immediate increase in the first session of conditioning. TIA in the ADp nucleus declined with the progress of training after the first conditioning session whereas the enhanced receptor binding was maintained throughout training. Binding of OXO and TIA in the AVm nucleus developed in parallel, exhibiting significant enhancement in the session in which criterion was reached. These increases of OXO binding were measured relative to binding in naive controls. The same patterns of increased OXO binding were found when the binding

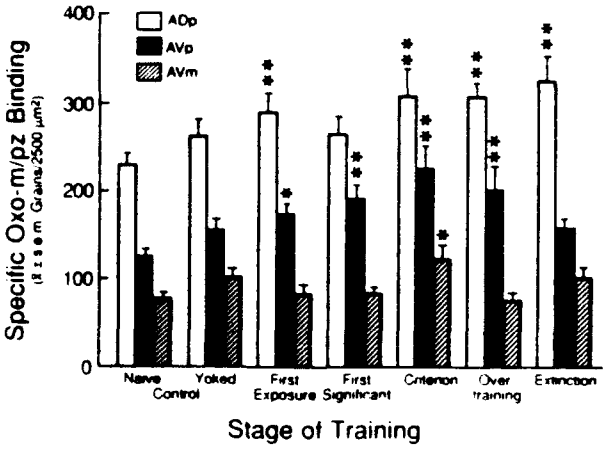

Figure 1. Binding of ^{3}H Oxotremorine co-incubated with Pirenzepine (OXO-M/PZ) throughout the course of training in three thalamic nuclei. As in the case of training-induced activity (Figure 3), the earliest binding changes occurred in the AD nucleus (ADp, open columns) during the first exposure (FE) to conditioning. Also, in keeping with training-induced activity, binding in AVp (solid columns) increased progressively reaching a plateau as the learning criterion was attained and then declined as overtraining and extinction were administered. Binding in AVm (hatched columns) showed significant increase only in the criterial as did training-induced activity in AVm. *single stars*, $p<05$ compared to naive controls; *double stars*, $p<.01$ compared to naive controls. Error bars represent SEM.

in trained subjects was compared to that in the yoked controls, however, the number of significant differences was reduced in this instance, as mean OXO binding in the yoked controls was numerically, though not statistically, greater than in the naive controls.

c. OXO Binding in Posterior Cingulate Cortex. OXO binding increased significantly relative to that in naive and yoked controls in layers I - III and V of posterior cingulate cortex (area 29c) in the session of the first significant (FS) behavioral discrimination. The effect in layer Ia was maintained throughout training and overtraining, whereas it occurred only during the FS session in the deeper layers.

d. ^{3}H Muscimol Binding to $GABA_A$ Receptors in Limbic Thalamus. Muscimol binding in the ADp, ADm, AVm and AM nuclei was significantly reduced during the first exposure (FE) to conditioning, relative to binding in a group that received only preliminary training (PT) with CSs and explicitly unpaired footshock. As in all groups that received training, the FE group received the PT treatment on the day before the FE session. Thus the reduced muscimol binding during FE in this group was due to the conditioning contingency, i.e., the pairing of the CS+ tone with the footshock. Muscimol binding in the ADp and ADm nuclei "recovered" in groups given training to the criterion and overtraining stages, i.e., binding in these stages was significantly greater than during the FE session. A non-significant trend toward this recovery was present in the data of the AVm and AM nuclei.

e. Mu and delta opoid receptors. Significant training-induced changes in binding of mμ and delta opoid receptor ligands were found, as presented in the 1989 Annual Technical Report. These data were based on the second of two limbic thalamic series, trained in 1989. Dr. Vogt is currently working on confirming assays at Bowman-Gray for the first (1988) series.

f. NE and Serotonin Turnover. An approximately inverse relationship was found between serotonin and NE turnover in rostral cingulate cortex. These transmitters and

their metabolites were measured using HPLC in conjunction with 16 electrode coulometric detection (ESA corp, Bedford Mass). Turnover of serotonin was calculated as the ratio of its metabolite, 5-HIAA, to serotonin. The turnover of NE was measured as the ratio of MHPG to NE. Serotonin turnover was high in naive control and overtrained rabbits (.70 +/- .08) and it was reduced to about .54 +/- .03 in yoked control and in all other training groups. In contrast, NE turnover was low in naive controls and overtrained rabbits (about .25 +/- .02) whereas yoked control and PT rabbits had a ratio of about .33 +/- .02. The ratio attained a peak in the first conditioning session (.42 +/- .04). Furthermore, posterior cingulate cortex exhibited a significant elevation (.7 +/- .01) of MHPG during the first conditioning session (FE) relative to naive controls (.46 +/- .02) the value in the yoked control group was .6 +/- .035. A manuscript reporting these results (Vogt, Volicer, Schnepper and Gabriel) has been submitted to Experimental Brain Research.

2. Discussion of Receptor Binding Data.

The discovery of increased OXO binding in the anterior thalamic nuclei constitutes the first observation of a direct correlation of receptor changes with TIA in brain circuits having known relevance to mammalian behavioral learning. These results taken together with other recent findings suggest a model (Figure 2) of the mechanism accounting for anterior thalamic TIA and they open the way for additional analyses of this mechanism.

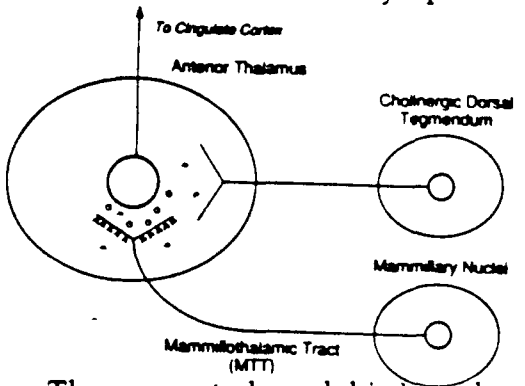

Figure 2. Hypothetical synaptic mechanism underlying training-induced neuronal plasticity in the anterior thalamus. M_2 muscarinic acetylcholine presynaptic receptors on mammillothalamic tract axon terminals are up-regulated during learning. Activation of these receptors by acetylcholine from the lateral dorsal tegmental nucleus (LDTN) during training enhances transmitter release and thus post-synaptic activation of anterior thalamic principal neurons.

The suggested model is based on results demonstrating that: a) anterior thalamic OXO binding increases during learning in correlation with TIA development; b) TIA is prevented[4] and M_2 receptors are lost[5] after lesions of the mammillothalamic tract; c) existing TIA is eliminated by systemic administration of scopolamine hydrobromide (Henzi, Kubota & Gabriel, 1990), and; d) cingulate cortical and hippocampal afferents to anterior thalamus are not involved in the production of thalamic TIA[7] (Gabriel et al., 1991). The essential proposition of the model is that TIA in the anterior thalamus is due to increased release of the mammillothalamic tract neurotransmitter. The increased transmitter release is mediated by the up-regulation of presynaptic M_2 auto-receptors on MTT axon terminals.

It is of great interest that muscimol binding was significantly reduced in the AD nucleus during the first session of conditioning. This reduction was correlated with massive excitatory TIA, unique to the AD nucleus, during the first exposure (FE) to conditioning, results which suggest that this AD thalamic excitatory TIA is due in part to down-regulation of $GABA_A$ receptors.

Binding in the PT session (which involved presentation of tones and unpaired shock) was significantly greater than binding in naive controls, whereas binding in the FE session was significantly less than in the naive controls, suggesting that the unpaired presentation of CS+ and shock in the PT session fostered up-regulation of $GABA_A$ receptors, and that the paired presentation of CS+ and shock in the FE session fostered an immediate down regulation. The increased muscimol binding the the PT condition is intriguingly concordant with prevalant views in the conditioning literature which regard the unpaired procedure as an inhibitory conditioning procedure. Inhibitory conditioning results from the negative contingency (the tone CSs are <u>never</u> followed by shock, and thus predict no-shock or "safety"). This safety conditioning thus gives rise to increased muscimol binding whereas "danger conditioning" induced by tone - shock pairing gives rise to decreased muscimol binding. Thus, $GABA_A$ receptors may be critically involved in the neural encoding of CS - shock contingencies, the learning of negative contingencies (the unpaired procedure) being associated with up-regulation and learning of positive contingencies (the paired procedure) being associated with down-regulation of these receptors. The fact that M_2 muscarinic receptors were significantly up-regulated in the AD nuclei indicates that both muscarinic and $GABA_A$ receptor dynamics are involved in the massive AD thalamic plasticity.

3. <u>Electrophysiological Studies (Objective I.B)</u>.

<u>a. Documentation of limbic thalamic excitatory TIA</u>. Objective I.B. involved documentation of TIA during conditioning and during presentation of unexpected stimuli in several limbic thalamic, cingulate cortical and hippocampal areas. These studies were intended to permit examination of the correspondences of TIA with receptor binding data and the documentation of learning-relevant circuit events and interactions. Here, the results of studies of limbic thalamic TIA are presented, as the limbic thalamic areas yielded the most abundant correspondences with the receptor binding data, as described above.

Data collection and analyses during the first two project years demonstrated TIA-development separately in several subdivisions of the anterior thalamus, the AD, AVp, AVm, the LD and the anterior medial (AM) nuclei. The records in all of these nuclei exhibited both excitatory and discriminative forms of TIA (Gabriel, Vogt, Kubota, Poremba & Kang, 1991). Excitatory TIA is the increase in the discharge magnitudes

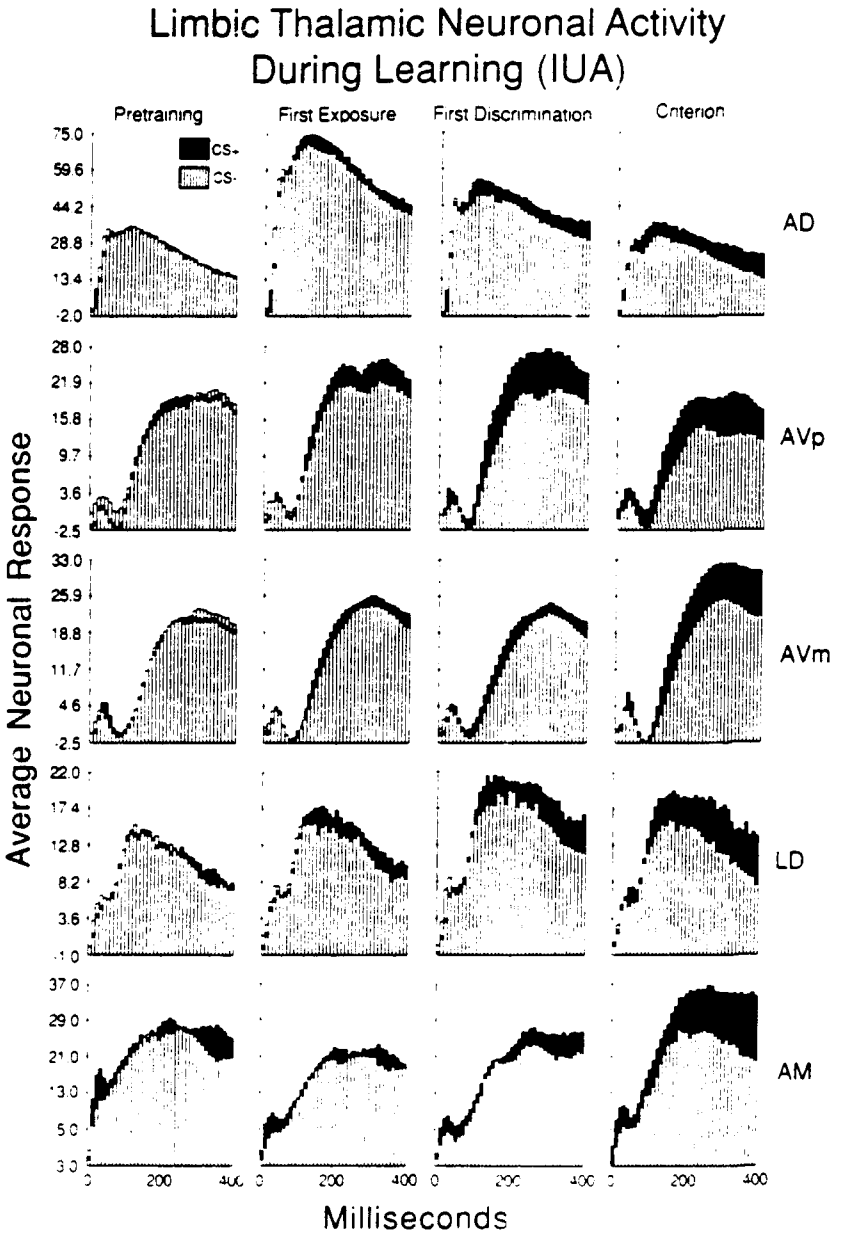

Figure 3. Neuronal activity in five nuclei of limbic thalamus during learning. The average integrated unit activity (IUA) profiles in the form of post stimulus histograms are plotted for the positive conditional stimulus (CS+, dark bars) and for the negative conditional stimulus (CS-, light bars) during the first exposure (FE) to the conditioning task, the session of the first significant (FS) behavioral discrimination between CS+ and CS-, and during the session in which the criterion (CRIT) of learning was attained. The plotted values represent discharge magnitudes normalized relative to a 300-millisecond pre-CS baseline. Onset of the CS is represented at "0" on the abscissae. Each panel shows the average discharge magnitude in 40 consecutive 10-millisecond intervals after CS onset. Data are shown for the anterior dorsal (AD), parvocellular anterior ventral (AVp), magnocellular anterior ventral (AVm), lateral dorsal (LD) and anterior medial (AM) nuclei of thalamus. Analyses of the data indicate that peak training-induced neurophysiological plasticity (TIA) in the AD nucleus occurs in the FE training stage, the TIA peaks in AVp and LD occur in the FS training stage, and the peaks in the AVm and AM nuclei occur in the CRIT session.

elicited by CS+ and CS- during training, compared to magnitudes elicited during PT with tones and unpaired shock. Discriminative TIA refers to the differential discharges in a particular session, i.e., the greater neuronal discharges elicited by the CS+ than by the CS-. All of the thalamic areas exhibited monotonically increasing discriminative TIA during learning. However, the pattern of excitatory TIA differed among the nuclei. Peak magnitude of excitatory TIA occurred in different training stages depending on the site of recording (Figure 2). AD neurons

exhibited maximum excitatory TIA in the first exposure (FE) to conditioning, AVp and LD records exhibited excitatory TIA in the session of the first significant (FS) behavioral discrimination, and AVm and AM records showed peak TIA in the session in which the criterion (CRIT) of learning was reached.

b. Discriminative TIA. These studies have not shown striking correlations between receptor regulation and discriminative TIA (i.e., the development of greater neuronal discharges to CS+ than to CS-) in limbic thalamus or in cingulate cortex, suggesting that discriminative TIA is not elaborated locally in the limbic structures but is instead elaborated at more peripheral sites and relayed to limbic thalamus. A likely candidate peripheral site of discrimination is the medial geniculate [MG] nucleus of auditory thalamus, a region in which discriminative TIA is found.[3] Preliminary data indicate abolition of CS elicited excitation in limbic thalamus following bilateral electrolytic lesions in the MG nucleus. If this result is confirmed the first definitive indication would be provided that limbic thalamic neurons receive auditory information from the MG nucleus, opening the way for studies of discriminative TIA in limbic thalamus with discrete fiber-sparing lesions in MG subnuclei (the medial and dorsal divisions of MG).

c. The CS Pathway. A problem with the suggestion that the MG nucleus provides discriminative TIA to the limbic thalamus concerns the fact that pathway tracing studies have failed to demonstrate direct projections from the MG nucleus to limbic thalamus. Thus, the route wherein information in the auditory thalamus accesses the limbic thalamus is unknown. Recent results are relevant to this issue: bilateral electrolytic lesions of the amygdaloid complex completely blocked behavioral acquisition and the development of excitatory and discriminative TIA in the AV thalamic nucleus (Poremba & Gabriel, 1991). These results suggest that information flows from auditory thalamus to the vicinity of the amygdala (a now well-documented pathway[9]) and from amygdala to limbic thalamus. The latter limb of this circuitous proposed pathway is also not direct, but instead involves a relay in the cholinergic tegmentum.[8]

d. Documentation of TIA during the presentation of unexpected stimuli: Analysis of limbic circuit interactions. The proposed electrophysiological studies under objective I, B were intended to test hypotheses[6] concerning the interactions of limbic cortical and thalamic circuitry mediating behavioral responses to unexpected events.

TIA in hippocampal subfields during learning. Multi-unit activity and field-potentials recorded during learning in three hippocampal subfields (dentate gyrus, CA1, CA3, posterior subiculum) have been described (Poremba et al., 1990). These data indicate development, in the first conditioning session, of excitatory TIA in all hippocampal subfields at the outset of conditioning. This rapid change declined and dissappeared rapidly as training continued past the first session. In addition, a gradual development of discriminative field-potential components occurred in subfield CA3 and the dentate gyrus. This discriminative

TIA did not attain significance until the criterial (CRIT) training session. The rapid development and fading of excitatory TIA indicates that components of hippocampal circuitry exhibit recency encoding, i.e., rapid tracking of new task contingencies. The gradually developing and persistant field potentials, on the other hand, indicate the occurrence of primacy encoding, i.e., the gradual and persistent encoding of the stable, time-invariant task features. The co-occurrence of these two forms of TIA in hippocampal circuitry is consistent with the hypothesis that hippocampal circuitry compares the primacy and recency mnemonic representations (Gabriel, 1990). The model proposes that a match of the two representations (meaning that recent/current events are the same as past events in the training situation) fosters a passive hippocampal role, allowing previously acquired behavior to be triggered by events in limbic thalamus and cingulate cortex. However, if mismatch occurs (meaning that the current inputs are unexpected in that they do not match expectancies based on memory), hippocampal outputs suppress behavior-inducing activity in limbic thalamus and cingulate cortex, instead invoking orienting/attentional responses, and memory updating.

Effects of unexpected stimuli. The original hypothesis[6] that gave rise to these studies proposed that novel events presented to trained rabbits are first detected by the hippocampal trisynaptic circuit, by virtue of the aforementioned comparison/mismatch processes. This detection then causes hippocampal outputs to act on limbic thalamus/cingulate cortex to prevent the conditioned response. Studies carried out under the aegis of objective I, B tested this hypothesis by recording unit activity in all limbic circuit areas in trained rabbits as they experienced extinction (CS presentation without shock). In one condition, a novel CS (of different auditory frequency than either the CS+ or CS-) was presented repeatedly. In a second condition, the standard CSs were presented with novel background (context) stimuli (reduced illumination and a novel wintergreen odor) in the conditioning chamber. Additional novelty treatments using standard training rather than extinction procedures included manipulation of the relative probabilities of CS+ and CS- presentations, and the scattered presentation of a novel CS among standard training trials. All conditions were presented to each rabbit in a counterbalanced manner.

The results, summarized below, are being prepared for publication.

In addition, studies of neuronal and behavioral responses to the novel events have been carried out in rabbits with hippocampal lesions. A preliminary report of the results has been presented (Kang et al., 1990).

Michael Gabriel

Conclusions based on studies pursuant to objective I, B, of neural circuit activities governed by unexpected stimuli in intact rabbits and in rabbits with hippocampal lesions, are listed in this section, with empirical documentation: 1) Consistent with the model, hippocampus is a critical epicenter necessary for novelty detection. This conclusion is based on the finding that hippocampal neurons exhibited the briefest latencies of novelty-specific discharges, relative to other monitored limbic cortical and thalamic areas. In addition, virtually all of the novelty-specific discharges of limbic thalamic and cingulate cortical neurons were eliminated in rabbits with hippocampal lesions; 2) Contrary to the model, the initial response of anterior thalamic and posterior cingulate cortical neurons to the novelty conditions presented during extinction was a modest increase or no change in cue-elicited discharge magnitude rather than the expected suppression of firing. Only after 20 to 40 presentations of the CS in novel extinction sessions did suppression of AV thalamic firing (relative to firing in a standard extinction session) emerge. The initial increases to the novel events occurred only in rabbits with intact hippocampi, indicating that the hippocampus maintains or increases AV thalamic activity when novelty is first encountered. The increased activity may represent increased attentional resources for the processing of the novel events. The increased activity in limbic thalamus occurred in correspondence with novelty-specific suppression of conditioned response performance. These findings call for a revision of the theoretical model. The revised model will recognize that suppression of brief-latency, cue-elicited AV thalamic and cingulate cortical activity is not a necessary condition for suppression of conditioned response output. It is proposed that cingulate cortical pre-motor discharges which occur in trained rabbits represent the activity that hippocampus must suppress in order to suppress conditioned responses. 3) The hippocampus is necessary for suppression of behavior in the novel context test but not for suppression of behavior in the novel CS test. In accord with extant theories stating that the hippocampus is involved in the encoding of multi-modal "configural" stimuli, alterations of contextual stimuli during extinction only suppressed responding in rabbits with intact hippocampi. Contextual alterations were not detected and consequently behavior was not rapidly suppressed during extinction in rabbits with hippocampal lesions. In contrast, the novel CS condition dramatically suppressed performance during extinction, relative to performance during extinction with the standard CS, and this suppression occurred both in intact rabbits and in rabbits with hippocampal damage. Thus the hippocampus is not needed for behavioral suppression induced by the novel CS (although, the neuronal data clearly indicated that the novelty of the novel CS was detected by hippocampal neurons). It is proposed that hippocampal suppression of the behavior in response to the novel CS was not needed as behavior was already amply suppressed by virtue of the fact that the novel CS had not been paired with the US and thus did not activate limbic thalamic and

cingulate cortical mnemonic circuits to elicit conditioned responses. These results neither affirm or contradict the theoretical model. Rather, they provide information that will foster a more elaborate and veridical updated model. A manuscript reporting these results is currently in preparation; 4) Limbic thalamic neuronal discharges and behavior are suppressed by reinforced novel stimuli. AV thalamic discharge magnitude was reduced in rabbits given reinforced training with novel CSs following training with standard CSs (Sparenborg et al., 1990; Stolar et al., 1989). These results are not at odds with the transient increments of AV thalamic activity reported above to occur at the outset of novel extinction, as the reduced thalamic activity was based on average neuronal data in an entire training session. Taken together these findings suggest that the very first presentation of novelty to a trained rabbit increases limbic thalamic firing. This increase depends on the hippocampus, and it enhances the neural analysis of the novel event. If the novel event is repeated without reinforcement (i.e., in an extinction test) the increased thalamic activity gives way to suppressed activty and behavioral extinction. However, if the novel stimulus is presented repeatedly as a CS+ (i.e., with reinforcement) and if the hippocampus is intact, the novel stimulus will suppress AV thalamic activity relative to the activity elicited under standard training conditions. This suppressed activity is to be expected, as the new stimuli have not been employed for an amount of training sufficient to permit the development of peak thalamic TIA. It is hypothesized that the AV thalamic discharge would eventually reach a TIA peak if sufficient training with the new stimulus is given. It is intriguing to imagine that discharges in other thalamic nuclei such as the AD nucleus, which exhibit early peaks of TIA, will be enhanced while the AV discharge will be suppressed, during training with a novel CS. This enhancement might reflect a "resetting" of the topographic pattern of anterior thalamic TIA peaks to a pattern that is appropriate to an earlier training stage, given the presentation of a new CS. These considerations suggest that the hippocampus is involved in maintaining an associatively appropriate set of anterior thalamic TIA peaks based on the amount of training history that has accrued for a particular stimulus.

These findings demonstrate how this project is elucidating the manner in which the hippocampus interacts with other structures of the limbic circuit to govern behavioral coping in response to unexpected events.

As mentioned, the foregoing results are being prepared for publication. However, this study will be continued, in order to determine whether the resetting of the thalamic peaks occurs when a new CS is introduced, and to provide a database for an empirical neural network analysis based on the intercorrelations of single neuron trains within the hippocampal subfields, and among each subfield and the cingulate cortical/limbic thalamic areas, during

standard conditioning and unexpected stimulus presentation.

4. Cholinergic deafferentation of the limbic thalamus induced by electrolytic and fiber sparing lesions of the lateral dorsal tegmentum (Objective III, C).

This study deals with the role of cholinergic afferents to limbic thalamus from the lateral dorsal tegmentum and related brainstem areas, in relation to limbic thalamic TIA and training-induced M_2 muscarinic ACh receptor binding. This project is of special importance in light of the key role in TIA development played by cholinergic projections to limbic thalamus, as indicated by studies described above.

Neuronal recordings during learning are being made in the anterior and MD thalamic nuclei in rabbits with unilateral and bilateral lesions of the tegmental cholinergic areas. Recordings are also being made in the tegmental areas in controls, in order to document the training-related firing patterns in regions containing cholinergic neurons. Initially, electrolytic lesions will be used. These are to be followed by fiber-sparing ibotenic acid lesions in the event that significant changes are noted. Sham lesions will be made in controls. It is anticipated on the basis of the results of systemic cholinergic receptor blockade and significant changes in M_2 receptors, that lesions of the cholinergic tegmentum will abolish TIA in the limbic thalamus. It will be of interest to note whether TIA in all limbic nuclei is governed by the tegmental projections, or whether a particular class of nuclei are affected. As the tegmental sites crucial for TIA development are identified, separate groups of rabbits with tegmental lesions, and sham controls, will recieve the standard battery of behavioral treatments followed by assay for M_2, $GABA_A$, and opiod receptors.

5. Cholinergic deafferentation of cingulate cortex induced by lesions of the diagonal band of Broca (DBB), cingulate cortical TIA and behavior.

Pursuant to objective III, B, 2, b This study delineates the contribution of the DBB projection to learning and cingulate cortical TIA. It is essentially complete and a preliminary report has been presented (Kubota et al., 1990). Manuscript submission is expected in 1992.

Standard acquisition followed by unexpected stimulus sessions were administered to rabbits with bilateral electrolytic DBB lesions and sham lesion controls with recording electrodes in the anterior and posterior cingulate cortex, AV and MD thalamic nuclei and hippocampal subfields CA1 and DG. The lesions were associated with significant depletion of acetylcholinesterase content in cingulate cortex based on light-densitomitry analysis of Timms stained coronal sections. In addition, significant performance deficits at all stages of behavioral learning occurred in rabbits with lesions, although the learning

criterion was attained by these rabbits. The brief-latency transient neuronal "on" discharge 20 milliseconds after CS onset was abolished both in anterior and posterior cingulate cortex, in rabbits with lesions. However, cingulate cortical excitatory and discriminative TIA developed normally in rabbits with the lesions. These results suggested that the cholinergic diagonal band projection to cingulate cortex is necessary for the brief latency "on" discharge, which may have an important organizing function, perhaps providing an initialization signal that contributes to timing of subsequent CS processing. Derangement of this process due to the lesions yields a generalized performance decrement in rabbits with lesions, but the lesions do not block learning or cingulate cortical plasticity. Thus, contrary to much theory, the cholinergic diagonal band projection does not seem to be involved in mnemonic processes of cingulate cortex. These results disaffirm the hypothesis that the DBB projection contributes importantly to plasticity-relevant changes in M_2 cingulate cortical acetylcholine receptors noted above.

6. Effects of lesions of the AD nucleus on discriminative avoidance behavior and on neuronal activity in cingulate cortex and the AV nucleus.

Past work has indicated that AD neurons exhibit an entirely unique pattern of TIA, a dramatic increase in cue-elicited firing in the very first conditioning session, relative to the preceding session with tone and noncontingent footshock presentation (Figure 2). This dramatic first-session excitatory TIA declines progressively in subsequent training sessions, whereas the TIA in the AV nucleus does not increase dramatically in the first session but rather rises gradually throughout the course of learning to criterion. The reciprocity between AD and AV TIA trajectories during acquisition suggested that AD neurons may be involved in the circuitry that is responsible for suppressing AV thalamic activity and behavior in the early stages of training. This suppression is viewed as a response to novelty, i.e., the rabbits' first experience of tone-shock pairings. As the novelty abates with further training the AD discharge subsides and the AV discharge, necessary for behavioral learning, increases. This hypothesis was tested by the proposed study (objective III, B, 5) of the effects of discrete lesions of the anterodorsal (AD) thalamic nucleus on cingulate cortical and AV thalamic TIA.

The study is now complete and the hypothesis has been substantially confirmed, as discrete electrolytic and ibotenic acid lesions restricted to the AD nucleus significantly increased the frequency of CR performance during stages of training in which novel events occurred (the first sessions of conditioning and extinction). The lesions also increased the magnitude of AV thalamic and cingulate cortical TIA. The manuscript reporting these findings is in the final stages of preparation, and will be submitted in the spring of 1992.

Michael Gabriel

7. Objectives involving the assessment of training-induced receptor regulation in posterior cingulate cortex

Several of the originally proposed objectives (III B: 1a, 2c, 3a. 3b, 4a, 4b) were pursuant to preliminary data indicating training-induced alterations of M_1 muscarinic receptor binding in the posterior cingulate cortex, obtained in an initial test series completed prior to the first project year. The preliminary findings did not hold up in three successive attempts to replicate which are described fully in the Annual Technical Report for 1989. Consequently, the related objectives were not pursued.

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Michael Gabriel

VI. PUBLICATIONS SUPPORTED BY AFOSR

Books

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Gabriel, M., Sparenborg, S., and Kubota, Y. Anterior and medial thalamic lesions, discriminative avoidance learning and cingulate cortical neuronal activity in rabbits. Experimental Brain Research, 1989, 76, 441-457.

Stolar, N., Sparenborg, S., Donchin, E., and Gabriel, M. Conditional stimulus probability and activity of hippocampal, cingulate cortical and limbic thalamic neurons during avoidance conditioning in rabbits. Behavioral Neuroscience, 1989, 103, 919-934.

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Poster Presentations with Published Abstract

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Michael Gabriel

VII. INTERACTIONS\COUPLING ACTIVITIES

A. Invited Addresses and Review Panel Membership

Invitation to participate in a Symposium entitled "The Orchestration of Memory: Systems, Synapses and Substrates", American Psychological Society 1992 Annual Meeting, San Diego CA, June, 2.

Invitation to participate in symposium on Neural Control of Movement, Marco Island, Florida, April 1991.

Neural and Connectionist Models of Avoidance Learning, research presentation, Illinois-Nijmegen Workshop on Computational Neuroscience, Beckman Institute, University of Illinois, September 1990, Urbana Illinois.

Neural Circuit Dynamics Underlying Learning: How Does Rabbit Know When to Run? Director's Seminar, Beckman Institute, University of Illinois, Urbana IL, November 1990.

Organizer of symposium entitled: Functional Relevance of the Brain's Cholinergic Projection Systems, Session of the January 1991 meeting of the Winter Conference for the Neurobiology of Learning and Memory, Park City Utah.

Cholinergic Receptor Regulation and Learning-Relevant Neuronal Plasticity in Rabbits. Invited presentation with Dr. B.A. Vogt at the January 1991 meeting of the Winter Conference on the Neurobiology of Learning and Memory, Park City Utah.

Limbic Circuit Interactions during Avoidance Learning; Research colloquium, Neuroscience and Behavior Program, Indiana University, Bloomington IN, Feb. 1990.

The Neurodynamics of avoidance learning, Research colloquium, Department of Psychology, Northwestern University, October 1989.

Neural Circuitry for Avoidance Learning, Invited address, Midwestern Psychological Association, May, 1990

Limbic System Interactions during Learning, research presentation, Midwestern Hippocampal Meeting, Northwestern University, July, 1989.

Michael Gabriel

Hippocampal Processing of Context, research presentation, Midwestern Hippocampal Meeting, Northwestern University, July, 1990.

Functions of Anterior and Posterior Cingulate Cortices During Learning in Rabbits; Research presentation, 16th Annual International Netherlands Institute for Brain Research Summer School; The Prefrontal Cortex, Amsterdam, August 1989.

Research presentation, Symposium on consensus regarding hippocampal function 14th Annual Winter Conference on Neurobiology of Learning and Memory, Park City, Utah, January 1988.

Membership, National Science Foundation Animal Learning and Behavior review panel, 1988-91.

B. Collaborative Projects and Facilities Expansion

Comparison of conditioning models. In addition to the collaboration with Dr. Vogt represented by the present project, a collaborative study with Dr. Joseph Steinmetz of Indiana University has demonstrated that different neural substrates mediate discriminative avoidance and nictitating membrane/eyelid conditioning in rabbits (Steinmetz, Sears, Gabriel, Kubota, Poremba & Kang, 1991). Lesions which abolish the oculomotor response do not affect the avoidance response.

A computational model. Developed in collaboration with Dr. Nestor A. Schmajuk of Northwestern University, a variant of other computational models that he as worked with sucessfully simulates acquisition and extinction of the avoidance CR in intact rabbits and in rabbits with hippocampal lesions (Gabriel & Schmajuk, 1990). The logical structure of the computational model is similar to the structure of our neurological model. We are now elaborating the computational model for use in generating and evaluating predictions concerning performance and neural circuit activities.

Multi-array recording. In collaboration with Dr. Bruce Wheeler of the Department of Electrical and Computer Engineering, the PI is working on the adaptation of fabricated silicon multi-array electrode probes, and other hardware and software approaches to the simultaneous recording of the activity of many single neurons in chronically-implanted behaving rabbits.

Visualization of Neuronal Intercorrelations during Information Processing in the Behaving Animal. This project has produced a vast number of simultaneously recorded

multi-unit and field potential records in several closely related areas of the brain during learning and in response to unexpected stimuli. Means are presently being explored to examine and quantify these interactions. One currently ongoing approach is being carried out in collaboration with Dr. Clint Potter (see Personnel below), who is providing instruction and appropriate modifications of a powerful new program (VIEWIT) enabling visualization of cross- correlations and other relationships among simultaneously recorded neuronal records, is being modified to permit direct application to the available database. The program will run on a powerful class of computers (e.g., SGI) especially adapted for high-resolution quasi 3-dimensional visualization of complex interrelations.

A new laboratory, facilities expansion, and a massive database for neural circuit interactions during learning.

The University of Illinois has established a new institute dedicated to the understanding of cognitive processes. Voted "Laboratory of the Year" by R&D Magazine in 1990, The Beckman Institute for Advanced Science and Technology is housed in a new $50,000,000. building, populated by several research groups including Artificial Intelligence, Cognitive Neuroscience, Complex Systems Research, Molecular Recognition, Neuronal Pattern Analysis, Physical Theory, Robotics, Statistical Analysis of Learning Networks, Supercomputing Applications and others. A successful intramural proposal submitted in 1987 by PI and several Neuroscientist colleagues resulted in the establishment of a Neuronal Pattern Analysis (NPA) Group in the Beckman institute, of which the PI is chairman. The PI moved his research to the Beckman Institute in the spring of 1989.

Funds made available by the University for the establishment of new laboratories in the Beckman Institute and equipment funds from a recently awarded NSF Center for the Neurobiology of Learning and Memory, in which PI is a core faculty participant, have been used to purchase two BrainWave Systems Inc. workstations, each with 800 megabyte optical disc drives. These workstations permit the use of principal components and other spike waveform parameters to extract single-cell information from the multi-unit records routinely recorded in this project. Spike extraction and intercorrelations among simultaneously recorded cells are now routine operations in the laboratory. We have established a new system including computer with interfaces for neuronal data collection and stimulus control, a completed data collection program, and a conditioning apparatus for appetitive (reward-based) learning. A new rotating-wheel conditioning apparatus has been constructed to replicate the avoidance conditioning system using PC-based hardware. Finally, a PC-compatible drive for .5-inch computer tapes has been purchased ($7000.) to transfer all of neuronal data, collected from 500 - 600 rabbits (6 brain areas and 15 - 20 training sessions for each rabbit) since 1982 to permanent archival storage on WORM optical disc. This is the first step toward

construction of a massive database system permitting 2-, and 3-dimensional graphic and numeric examination of average neuronal firing patterns and evoked field potentials during all stages of conditioning and testing, for any sequence of conditioning trials and events, in the many brain areas that we have studied including cingulate cortical layers, hippocampal subfields and limbic and auditory thalamic subnuclei.

An important step toward the goal of implementing a massive database system for the project data has recently been taken in the form of a very favorable review by the NSF Program in Biological Instrumentation, of a proposal written by Dr. Gabriel entitled "A Database System for Neuronal Pattern Analysis" (Dr. Gabriel=PI). This project provides programming, hardware and software for the implementation of a powerful relational database system for processing of neurophysiological time series data, including software for analysis and visualization of neurophysiological data and intercorrelations, and special provisions for sharing of data with researchers in other laboratories. All of the members of the Beckman Institute Neuronal Pattern Analysis Group are participants in this project. The NSF Program Manager has stated on December 27th of 1991 that the Program would like to "fund this project with enthusiam", and the members of the Beckman group currently await final action.

VIII. PARTICIPATING PROFESSIONALS

Dr. Michael Gabriel
Principal Investigator, Ph.D., Professor, Department of Psychology and Beckman Institute, University of Illinois, Urbana\Champaign (UIUC).

Dr. Brent A. Vogt
Principal Investigator, Ph.D., Associate Professor, Department of Physiology and Pharmacology, Bowman-Gray Medical School, Winston-Salem North Carolina.

Dr. Bruce Wheeler
Dr. Wheeler is an Associate Professor in the UIUC Department of Electrical and Computer Engineering. Dr. Wheeler is collaborating with PI in working out techniques for multi-array recording of neuronal activity during learning and for analysis and visualization of intercorrelations among simultaneously recorded neuronal activities in the limbic circuit.

Dr. Clint Potter
Mr. Potter is an Image Processing Specialist employed by the UIUC NSF National Center for Supercomputing Applications and the Biomedical Magnetic Resonance Laboratory of the UIUC College of Medicine. Mr. Potter is providing to the project consultation on

the use of his software (Program Name = VIEWIT) for visualization of intercorrelations among simulaneously recorded limbic circuit neuronal activities during learning.

Dr. Nestor A. Schmajuk
Dr. Schmajuk is an assistant professor in the Department of Psychology at Northwestern University. He recently collaborated with Dr. Gabriel to produce a mathematical counterpart of the theoretical model that guides project studies. The model is described in a recent chapter (Gabriel & Schmajuk, 1990) listed above.

Amy Poremba
Mrs. Poremba is a fourth-year graduate research assistant in the UIUC Biological Psychology Program supported by a teaching assistantship in Biological Psychology. She is currently working on several laboratory projects and beginning a dissertation on the contribution of the amygdala to limbic thalamic TIA.

Eunjoo Kang
Ms. Kang earned an M.A. in Physiological Psychology at the University of Seoul and is currently a fourth-year graduate research assistant in the UIUC Biological Psychology Program supported by a teaching assistantship in Biological Psychology. She is currently working on several laboratory projects and beginning a dissertation on the interactions of hippocampal and limbic thalamic circuits during learning and during processing of unexpected events.

Yasuo Kubota
Mr. Kubota earned his B.A. at Western Illinois University and is currently a fifth-year graduate research assistant in the UIUC Biological Psychology Program supported by a training stipend from the NSF Center for the Neurobiology of Learning and Memory. He is currently working on several laboratory projects and beginning a dissertation on cholinergic mechanisms of limbic thalamic single unit training-induced activity studied via thalamic cholinergic deafferentation induced by dorsal tegmental lesions.

David Tcheng
Mr. Tcheng is a fourth-year graduate student in the Department of Computer Science who has written important project programs and collaborated with Dr. Gabriel on production of a connectionist neural network model of limbic circuit leaerning-relevant interactions. He was supported by AFOSR (50%) and by NIH (50%) funds.

Regina Winfrey
Ms. Winfrey was a full-time laboratory technician responsible for daily data collection, scheduling and organizing daily laboratory activities. She was supported by AFOSR (50%) and by NIH grant to MG (50%), but was recently released due to non-renewal of project funds.

Michael Gabriel

Sun Jing
Ms. Jing was a graduate student in the Department of Statistics, providing 50% technical assistance involving programming and data analysis. She was released due to non-renewal of project funds.

Nick Kisseberth
Mr. Kisseberth was an undergraduate computer science major and programmer supported during the summer of 1990, 25% by AFOSR project funds and 25% by an NIH grant to MG. He is now supported by Beckman Institute Funds for work unrelated to the project.

Thomas Kessler
Mr. Kesslear is a UIUC Department of Psychology electronic hardware technician who has repaired and fabricated electronic circuits for the project.

Joseph Cobb
Mr. Cobb is a Beckman Institute electronic hardware technician who has repaired and fabricated electronic circuits for the project.

Judy Whittingham
Mrs. Whittington is UIUC Beckman Institute secretary who provides 25% effort to Dr. Gabriel.

Barbara Hartman
Mrs. Hartman is the UIUC Department of Psychology Business Manager who oversees accounting of project funds

Carol Ericksen
Mrs. Ericksen is a UIUC Department of Psychology secretary who oversees purchasing with project funds

Brenda Holtoff
Mrs. Holtoff is a UIUC Department of Psychology Secretary who keeps records of project expenditures and provides monthly budget reports.

Mae Donaldson
Ms. Donaldson is a UIUC Beckman Institute business secretary oversees expenditures of project-relevant Beckman Institute accounts.